Reconstruction of the extinct Ezo wolf's diet

Short title: Feeding habits of the extinct Ezo wolf

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Abstract

On Hokkaido, Japan, the Ezo wolf (*Canis lupus hattai*), an apex predator, became extinct at the end of the 19th century owing to human activities. Top predators often have an important role in their ecosystems, yet we have no scientific information on the feeding habits of the Ezo wolf. We performed carbon and nitrogen stable isotope analysis and radiocarbon dating of specimens of the wolf (n = 7) and its prey species and estimated the components of the wolves' diet using an isotope mixing model. Radiocarbon dating suggested that most of the wolves examined came from different populations or generations. The mean stable isotope ratios of the wolves were −19.5 ‰ (± 1.9 ‰ SD) for δ^{13}C and 8.7 ‰ (± 2.6 ‰ SD) for δ^{15}N. The discrimination-corrected isotopic ratios of five of the seven wolves were almost the same as those of Sika deer at the same sites. In contrast, those of two wolves had clearly higher isotopic values than those of deer, suggesting that these wolves depended partly on marine prey such as salmon and marine mammals. Thus, Ezo wolves had similar ecological roles to Canadian grey wolves, and were a second subspecies shown to have fed on a marine diet, in addition to the "coastal wolves" of British Columbia.

Key words: dietary reconstruction, extinct mammal, *Canis lupus*, stable isotope, marine prey

Introduction

Wolf species are distributed widely in the Northern Hemisphere and are typical apex predators in terrestrial ecosystems. Wolves feed primarily on terrestrial mammals such as ungulates and rodents (e.g., Ciucci, Tosoni & Boitani 2004, Morehouse & Boyce 2011). However, recent studies show that grey wolves on the central coast of British Columbia, Canada, uniquely depend heavily on marine prey. Unlike interior populations, these "coastal wolves" (Darimont, Reimchen & Paquet 2003) have a distinct genetic structure and distinct feeding habits (Stronen et al. 2014). The recognition of these coastal wolves is an important key to understanding drivers of genetic differentiation among neighbouring populations.

Hokkaido, at the northern end of Japan, was inhabited by a subspecies of grey wolf, the Ezo wolf (*Canis lupus hattai*), until the end of the 19th century, when it became extinct owing to human activities (Inukai 1982). This was the first human-caused extinction of a mammal species in Japan. Because Ezo wolves disappeared before their ecology could be investigated, there is almost no information about their past ecological roles. In particular, their feeding habits are important for understanding their ecological roles. Umeki (2016) found no scientific information about their feeding habits in the literature. As Hokkaido is similar to Canada in both environmental conditions and fauna, various marine animals, such as anadromous salmon species (*Oncorhynchus* spp.) and the carcasses of marine mammals, could have been prey items for Ezo wolves, as they are in Canada. Therefore, if Ezo wolves fed on marine animals as well as terrestrial ones, we might detect such a diet by stable isotope analysis of their bones (e.g. Hilderbrand et al. 1996, Matsubayashi et al. 2015).

Here, we aimed at reconstructing the feeding habits of Ezo wolves by using stable isotope analysis of bone collagen. Ezo wolves and domestic dogs in Hokkaido are distinguishable by their skeletal size (Ishiguro et al. 2010). Bones excavated from archaeological sites in Hokkaido have been identified by archaeologists and reported. Few bone fragments of the

Ezo wolf have been excavated, but we searched for as many as we could find in the archaeological reports available at the Hokkaido Archaeological Operations Center, and obtained samples. We measured radiocarbon values and stable carbon and nitrogen isotope ratios in the bone collagen of the samples and compared the isotopic values with those of potential prey species to reconstruct the wolves' feeding habits and differences among individuals. Investigating their feeding habits, particularly the potential consumption of a marine diet, is the first step in understanding the ecological role of Ezo wolves in past ecosystems.

Materials and methods

Study site

This study was conducted on Hokkaido, the northernmost island of Japan (Fig. 1). The western half of the island is in the cool temperate zone and the eastern half is subarctic. Sika deer are distributed across the island, mainly in the central and eastern areas (Yamamura et al. 2008). Chum salmon (*Oncorhynchus keta*) run from October to February in the western and eastern areas, and pink salmon (*Oncorhynchus gorbuscha*) run from August to October in the east.

Sample collection and stable isotope analysis

We collected bone fragments of wolves (n = 8) and their potential mammal prey species from local museums (Fig. 1, Table 1), but we used bones of modern salmon (bought at the market in eastern Hokkaido in 2012) because salmon bones are rarely excavated from archaeological sites. All of the wolves used were confirmed as adults, because skeletons of adult wolves, unlike those of young wolves, are distinguishable from those of domestic dogs.

Samples of bone collagen were extracted according to the methods of Schoeninger & DeNiro (1984), and their carbon and nitrogen stable isotope values were measured by mass spectrometry. Stable isotope ratios are expressed in δ notation in accordance with the international standard scale, based on the following equation (Bond & Hobson 2012):

$$\delta X = (R_{\text{sample}} / R_{\text{standard}}) - 1, \quad (1)$$

where X is ^{13}C or ^{15}N; R_{sample} is the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio of the sample; and R_{standard} is that of Vienna Pee Dee Belemnite for $^{13}C/^{12}C$ or of atmospheric nitrogen for $^{15}N/^{14}N$. The stable isotope ratios were measured on a Delta XP mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) connected to a Flash EA 2000 elemental analyser (Thermo Fisher Scientific) at the Research Institute for Humanity and Nature (Kyoto, Japan). The elemental concentrations and isotope ratios of carbon and nitrogen were calibrated against alanine ($\delta^{13}C$ = −19.04 ‰, $\delta^{15}N$ = 22.71 ‰) and glycine ($\delta^{13}C$ = −34.92 ‰, $\delta^{15}N$ = 2.18 ‰) laboratory standards (Tayasu et al. 2011), which are traceable back to the international standards. The analytical standard deviations (SD) of these standards were 0.05 ‰ ($\delta^{13}C$) and 0.11 ‰ ($\delta^{15}N$) for alanine (n = 6) and 0.02 ‰ ($\delta^{13}C$) and 0.05 ‰ ($\delta^{15}N$) for glycine (n = 6).

Correction of isotope values in modern salmon bone collagen samples

We corrected the stable isotope ratios of modern salmon for the expected changes in isotopic values over time. Carbon stable isotope ratios show a historical decrease after 1860 due to human consumption of fossil fuels (Suess effect). Ice-core studies (Francey et al. 1999, Chamberlain et al. 2005) show that atmospheric $\delta^{13}C$ decreased by 0.05 ‰ per decade between 1860 and 1960 and by 0.22 ‰ per decade since 1960 (Wiley et al. 2013). Therefore, we increased the $\delta^{13}C$ of the modern salmon by 1.60 ‰. The $\delta^{15}N$ value is also expected to have changed historically owing to anthropogenic atmospheric nitrogen and biological nitrogen fixation (the conversion of N_2 gas to ammonium). For marine prey, the maximum

106 change in δ^{15}N resulting from anthropogenic atmospheric nitrogen is 0.03 ‰, and that
107 resulting from N fixation is 0.13 ‰ (Wiley et al. 2013). Therefore, we reduced the δ^{15}N of
108 modern salmon by 0.16 ‰. We used these corrected isotopic values in the subsequent
109 statistical analyses.
110

111 **Mixing model and statistical analyses**

112 Prey items were initially categorized as terrestrial mammals, anadromous salmon, or marine
113 mammals. We used a *K* nearest-neighbour randomization test (Rosing, Ben-David & Barry
114 1988) to investigate whether the stable isotope ratios of the various food types differed
115 significantly from each other, and all diet items were isotopically segregated (Bonferroni
116 adjusted $P < 0.01$). Then we evaluated the proportional contribution of each food resource to
117 the wolves' diet using Stable Isotope Analysis in R (SIAR), a Bayesian isotopic mixing model
118 available as an open-source R package (Parnell et al. 2010). The SIAR Markov chain Monte
119 Carlo algorithm was run for 1 000 000 iterations. The first 100 000 samples were discarded to
120 avoid the possibility of starting value effects. We also assigned elemental concentration
121 values of prey items (%C and %N in Table 1) to account for the differences in C and N
122 concentrations (Phillips & Koch 2002). Because different tissues incorporate isotopes at
123 different rates, we applied a correction factor for bone collagen to incorporate the isotopic
124 discrimination (Trophic Discrimination Factor: TDF) between predator and prey before
125 generating the model. On the basis of several published studies that described wolf–prey
126 isotopic TDF, we set an isotopic TDF of 1.1 ‰ ± 0.2 ‰ SD for δ^{13}C (Szepanski et al. 1999,
127 Bocherens & Drucker 2003, Fox-Dobbs 2007) and 3.9 ‰ ± 1.1 ‰ SD for δ^{15}N (Schwarcz &
128 Schoeninger 1991, Szepanski et al. 1999, Bocherens & Drucker 2003, Fox-Dobbs 2007). All
129 statistical analyses were conducted in R (R Core Team 2013).
130

Radiocarbon dating

We used radiocarbon (^{14}C) dating to determine the year of death of the ancient wolves, but not of the modern wolves (HUB9880 and HUB9890B), for which accurate information is recorded. We obtained enough collagen from two ancient wolves (TN1-01, WO-01) to graphitize the carbon in it, but not for the other three (OPA-01, UEN-01 and YNG-01). For UEN-01 and YNG-01, we used bone collagen of a deer (UEN-03 and YNG-02) which was excavated with the wolf. The age of OPA-01 was determined from geological information at the site (BBE 1997).

Collagen samples used for carbon and nitrogen stable isotope analysis were also used for the radiocarbon analysis. Targets were graphitized at the Research Institute for Humanity and Nature, and ^{14}C concentrations were measured by accelerator mass spectrometry at the Institute of Accelerator Analysis Ltd (Kanagawa, Japan). ^{14}C results are expressed as a percentage of modern (1950) carbon, which is corrected for isotopic discrimination using the $\delta^{13}C$ values. The ^{14}C dates were calibrated against the IntCal13 calibration curve (Reimer et al. 2013) in OxCal v. 4.2 software (Ramsey 2009).

Results

One of the wolf collagen samples (TKR-01; Table 1) showed a higher C/N ratio (8.9) than the others, well outside the established range of pure bone collagen samples (2.9–3.6; DeNiro 1985), so its isotopic data were not used in further analyses. The mean stable isotope ratios of the wolves were −19.5 ‰ ± 1.9 ‰ SD (range, −21.0 ‰ to −15.2 ‰) for $\delta^{13}C$ and 8.7 ‰ ± 2.6 ‰ SD (4.5 ‰–13.7 ‰) for $\delta^{15}N$. The discrimination-corrected isotopic ratios of five of the seven wolves were almost the same as those of Sika deer at the same sites (Fig. 2). In contrast, those of YNG-01 and UEN-01 were higher than those of deer (Fig. 2). The results of

the mixing model analysis suggested that these two wolves ate some marine prey; however, wide range of 95 % credible intervals showed that our model could not successfully dissect the proportions of salmon and marine mammals (Table 2).

^{14}C dating showed that the wolves TN1-01, WO-01, and YNG-01 and the deer UEN-03 died between 3961 and 3106 BC (Table 3). Archaeological records showed that OPA-01 died between AD 1667 (the date of eruption of Mt Tarumae, in the volcanic ash layer of which the remains were found) and AD 1913 (when the site was found) (BBE 1997).

Discussion

The results of radiocarbon dating suggested that most of the isotopic data were derived from different wolf populations or generations and were not biased to particular populations, although WO-01 and YNG-01 were not temporally isolated. Therefore, the variation in stable isotope values of the wolves derives from both population-level and individual-level differences in feeding habits. The ratios were clearly different from those of domestic dogs (Fig. 2), so these animals were not large domestic dogs.

A recent genetic study of the Ezo wolf showed that its mitochondrial DNA was identical to that of the Canadian grey wolf (Ishiguro et al. 2010). Canadian grey wolves show a variety of feeding habits at both the population and individual levels. For instance, wolf populations of coastal British Columbia have three different feeding habits: mainland wolves feed mostly on terrestrial resources, Outer Islands subpopulations depend strongly on marine resources, and Inner Islands subpopulations feed on both resources (Darimont et al. 2009). Our stable isotope results showed that Ezo wolves likely had feeding habits that were similar to those of the Inner Islands subpopulation in British Columbia, because both populations depend on both marine and terrestrial prey.

Several dietary studies of ancient domestic dogs on Hokkaido showed that all of these

dogs fed on marine prey provided by humans (Fig. 2; Naito et al. 2010, Tsutaya et al. 2014). On the other hand, the δ^{15}N values of the wolves in this study were clearly distinct from that of domestic dogs, suggesting that the wolves were not fed by humans. However, we cannot exclude the possibility that UEN-01 and YNG-01 sometimes fed on marine prey provided by humans. Further analysis to reconstruct the isotope chronology of individual mammals, such as incremental stable isotope analysis in dentine (Beaumont et al. 2013), should help to answer the question.

Although wolves can feed on carcasses of marine mammals (e.g. Darimont & Paquet 2002, Watts et al. 2010), grey wolves in Canada feed primarily on spawning salmon (Darimont et al. 2004). Stable isotope analysis suggested that the Ezo wolves also ate more salmon than marine mammals (Fig. 2). However, YNG-01 is likely to have fed on some marine mammals (Table 2), which could have been available to some wolf populations on Hokkaido. Consumption of marine organisms by terrestrial mammals is an important route of transfer of marine-derived nutrients to terrestrial ecosystems (e.g. Hilderbrand et al. 1999, Helfield & Naiman 2006). Thus, some wolf populations in coastal areas of Hokkaido could have had a role in transporting marine-derived nutrients to terrestrial ecosystems, unless humans were feeding them.

To date, the existence of wolves with a high dependence on marine prey has been reported only on the North American coast (e.g. Szepanski et al. 1999, Darimont & Reimchen 2002, Darimont et al. 2009, Watts et al. 2010). Our data suggest that the Ezo wolf also fed on a marine diet. The coastal wolves in British Columbia are considered an evolutionarily significant unit (Ryder 1986), which is valuable for conservation because of its genetic and ecological uniqueness (Muñoz-Fuentes et al. 2009). This implies that Ezo wolves, with their partial dependence on marine prey, were important for terrestrial ecosystems on Hokkaido.

Competing interests

We have no competing interests.

Authors' contributions

J.M. designed the study; O.T. helped acquire the specimens; J.M., T.O. and I.T. performed the chemical and stable isotope analyses. All authors approved the manuscript for publication.

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References

Beaumont, J., Gledhill, A., Lee-Thorp, J. & Montgomery, J. (2013). Childhood diet: a closer examination of the evidence from dental tissues using stable isotope analysis of incremental human dentine. *Archaeometry.* 55**,** 277–295.

Bocherens, H. & Drucker, D. (2003). Trophic level isotopic enrichment of carbon and nitrogen in bone collagen: case studies from recent and ancient terrestrial ecosystems. *Int. J. Osteoarchaeol.* **13,** 46–53.

228 Bond, A.L. & Hobson, K.A. (2012). Reporting stable-isotope ratios in ecology: recommended
229 terminology, guidelines and best practices. *Waterbirds*, **35,** 324–331.
230 BBE (Biratori Board of Education). (1997). *Cultural property investigation report of*
231 *Opaushinai 1 site related to a construction of Biratori bypass (National road No. 237).*
232 Biratori, Hokkaido, Japan (in Japanese).
233 Chamberlain, C.P., Waldbauer, J.R., Fox-Dobbs, K., Newsome, S.D., Koch, P.L., Smith,
234 D.R., Church, M.E., Chamberlain, S.D., Sorenson, K.J. & Risebrough, R. (2005).
235 Pleistocene to recent dietary shifts in California condors. *Proc. Natl. Acad. Sci. USA.* **102,**
236 16707–16711.
237 Ciucci, P., Tosoni, E. & Boitani, L. (2004). Assessment of the point-frame method to quantify
238 wolf *Canis lupus* diet by scat analysis. *Wildl. Biol.* **10,** 149–153.
239 Darimont, C.T. & Paquet, P.C. (2002). The gray wolves, Canis lupus, of British Columbia's
240 Central and North Coast: distribution and conservation assessment. *Can. Field Nat.* **116,**
241 416–422.
242 Darimont, C.T. & Reimchen, T.E. (2002). Intra-hair stable isotope analysis implies seasonal
243 shift to salmon in gray wolf diet. *Can. J. Zool.* **80,** 1638–1642.
244 Darimont, C.T., Reimchen, T.E. & Paquet, P.C. (2003). Foraging behaviour by gray wolves
245 on salmon streams in coastal British Columbia. Can. J. Zool. **81,** 349–353.
246 Darimont, C.T., Price, M.H.H., Winchester, N.N., Gordon-Walker, J. & Paquet, P.C. (2004).
247 Predators in natural fragments: foraging ecology of wolves in British Columbia's central
248 and north coast archipelago. *J. Biogeogr.* **31,** 1867–1877.
249 Darimont, C.T., Paquet, P.C. & Reimchen, T.E. (2009). Landscape heterogeneity and marine
250 subsidy generate extensive intrapopulation niche diversity in a large terrestrial vertebrate.
251 *J. Anim. Ecol.* **78,** 126–133.
252 DeNiro, M.J. (1985). Postmortem preservation and alteration of in vivo bone collagen isotope

253 ratios in relation to palaeodietary reconstruction. *Nature*, **317,** 806–809.
254 Fox-Dobbs, K., Bump, J.K., Peterson, R.O., Fox, D.L. & Koch, P.L. (2007). Carnivore-
255 specific stable isotope variables and variation in the foraging ecology of modern and
256 ancient wolf populations: case studies from Isle Royale, Minnesota, and La Brea. *Can. J.*
257 *Zool.* **85,** 458–471.
258 Francey, R.J., Allison, C.E., Etheridge, D.M., Trudinger, C.M., Enting, I.G., Leuenberger, M.,
259 Langenfelds, R.L., Michel, E. & Steele, L.P. (1999). A 1000-year high precision record
260 of δ^{13}C in atmospheric CO2. *Tellus B: Chem. Phys. Meteorol.* **51,** 170–193.
261 Helfield, J.M. & Naiman, R.J. (2006). Keystone interactions: salmon and bear in riparian
262 forests of Alaska. *Ecosystems*. **9,** 167–180.
263 Hilderbrand, G.V., Farley, S.D., Robbins, C.T., Hanley, T.A., Titus, K. & Servheen, C.
264 (1996). Use of stable isotopes to determine diets of living and extinct bears. *Can. J. Zool.*
265 **74,** 2080–2088.
266 Hilderbrand, G.V., Thomas, A.H., Charles, T.R. & Charles, C.S. (1999). Role of brown bears
267 (*Ursus arctos*) in the flow of marine nitrogen into a terrestrial ecosystem. *Oecologia*. **121,**
268 546–550.
269 Inukai, T. (1982). Eradication of Ezo wolf by humans. *Zenshu Nihon Doubutsushi.* **5,** 11–15
270 (in Japanese).
271 Ishiguro, N., Inoshima, T., Shigehara, N., Ichikawa, H. & Kato, M. (2010). Osteological and
272 genetic analysis of the extinct Ezo wolf (*Canis lupus hattai*) from Hokkaido Island,
273 Japan. *Zool. Sci.* **27,** 320–324.
274 Matsubayashi, J., Morimoto, J.O., Tayasu, I., Mano, T., Nakajima, M., Takahashi, O.,
275 Kobayashi, K. & Nakamura, F. (2015). Major decline in marine and terrestrial animal
276 consumption by brown bears (*Ursus arctos*). *Scientific reports*, **5,** 9203.
277 Morehouse, A.T. & Boyce, M.K. (2011). From venison to beef: seasonal changes in wolf diet

278 composition in a livestock grazing landscape. *Front. Ecol. Environ.* **9,** 440–445.
279 Muñoz-Fuentes, V., Darimont, C.T., Wayne, R.K., Paquet, P.C. & Leonard, J. A. (2009)
280 Ecological factors drive differentiation in wolves from British Columbia. *J. Biogeogr.* **36,**
281 1516–1531.
282 Naito, Y.I., Chikaraishi, Y., Ohkouchi, N., Mukai, H., Shibata, Y., Honch, N.V., Dodo, Y.,
283 Ishida, H., Amano, T., Ono, H. & Yoneda, M. (2010). Dietary reconstruction of the
284 Okhotsk culture of Hokkaido, Japan, based on nitrogen composition of amino acids:
285 implications for correction of ^{14}C marine reservoir effects on human bones, *Radiocarbon*
286 **52,** 671–681.
287 Parnell, A. C., Inger, R., Bearhop, S. & Jackson, A. L. (2010). Source partitioning using
288 stable isotopes: coping with too much variation. *PloS one*. **5,** e9672.
289 Phillips, D.L. & Koch, P.L. (2002). Incorporating concentration dependence in stable isotope
290 mixing models. *Oecologia.* **130,** 114–125.
291 R Core Team. (2013). R: A language and environment for statistical computing. R Foundation
292 for Statistical Computing, Vienna, Austria.
293 Ramsey, C.B. (2009). OxCal Program v4.2. http://www.rlaha.ox.ac.uk/orau/oxcal.html
294 (accessed 11 October 2016).
295 Reimer, P.J., Bard, E., Bayliss, A., Beck, J. W., Blackwell, P. G., Ramsey, C. B., Grootes, P.,
296 Guilderson, T., Haflidason, H., Hajdas, I., et al. (2013). IntCal13 and Marine13
297 radiocarbon age calibration curves 0–50 000 years cal BP. *Radiocarbon*. **55,** 1869–1887.
298 Rosing, M.N., Ben-David, M. & Barry, R.P. (1988). Analysis of stable isotope data: a K
299 nearest-neighbours randomisation test. *J. Wildl. Manage.* **62,** 380–388.
300 Schoeninger, M. J. & DeNiro, M. J. (1984). Nitrogen and carbon isotopic composition of
301 bone collagen from marine and terrestrial animals. *Geochim. Cosmochim. Acta* **48,** 625–
302 639.

303 Schwarcz, H.P. & Schoeninger, M.J. (1991). Stable isotope analyses in human nutritional
304 ecology. *Am. J. Phys. Anthropol.* **34,** 283–321.
305 Stronen, A. V., Navid, E. L., Quinn, M. S., Paquet, P. C., Bryan, H. M. & Darimont, C. T.
306 (2014). Population genetic structure of gray wolves (*Canis lupus*) in a marine archipelago
307 suggests island-mainland differentiation consistent with dietary niche. *BMC ecol.* **14,** 1.
308 Szepanski, M.M., Ben-David, M. & Ballenberghe, V.V. (1999). Assessment of anadromous
309 salmon resources in the diet of the Alexander Archipelago wolf using stable isotope
310 analysis. *Oecologia.* **120,** 327–335.
311 Tayasu, I., Hirasawa, R., Ogawa, N.O., Ohkouchi, N. & Yamada, K. (2011). New organic
312 reference materials for carbon- and nitrogen-stable isotope ratio measurements provided
313 by Center for Ecological Research, Kyoto University, and Institute of Biogeosciences,
314 Japan Agency for Marine-Earth Science and Technology. *Limnology*. **12,** 261–266
315 Tsutaya, I., Naito, Y.I., Ishida, H. & Yoneda, M. (2014). Carbon and nitrogen isotope
316 analyses of human and dog diet in the Okhotsk culture: perspectives from the Moyoro
317 site, Japan. *Anthropol. Sci.* **122,** 89–99.
318 Umeki, K. (2016). Research history of extinct wolves in Hokkaido: Overview and issue.
319 *Research Journal of Graduate Students of Letters*. **15,** 35–67 (in Japanese).
320 Watts, D. E., Butler, L. G., Dale, B. W. & Cox, R. D. (2010). The Ilnik wolf Canis lupus
321 pack: use of marine mammals and offshore sea ice. *Wildl. Biol.* **16,** 144–149.
322 Wiley, A.E., Ostrom, P.H., Welch, A.J., Fleischer, R.C., Gandhi, H., Southon, J.R., Stafford,
323 T.W., Penniman, J.F., Hu, D., Duvall, F.P. & James, H.F. (2013). Millennial-scale
324 isotope records from a wide-ranging predator show evidence of recent human impact to
325 oceanic food webs. *Proc. Natl. Acad. Sci. USA.* **110,** 8972–8977.
326

Figure legends

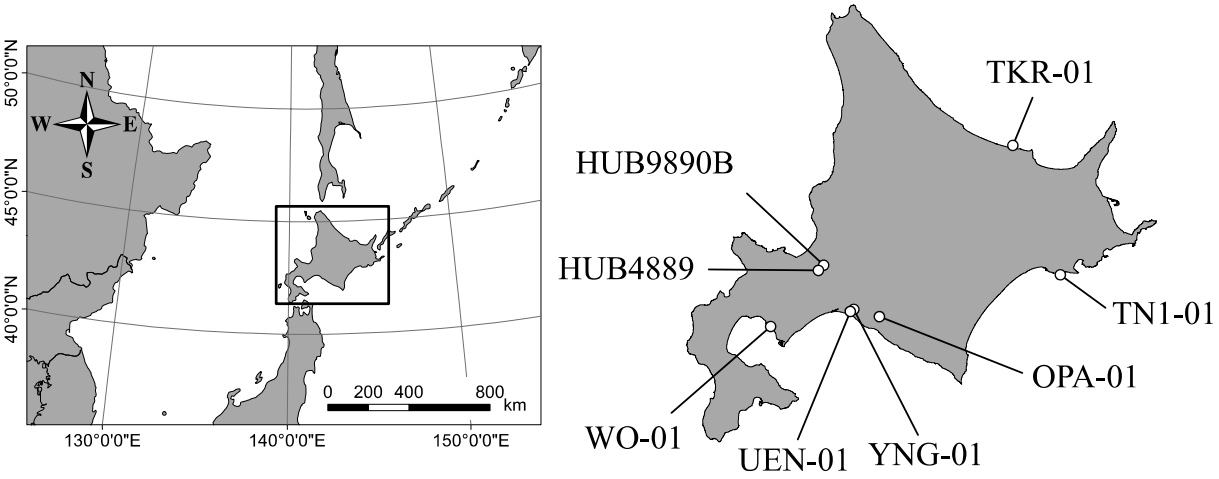
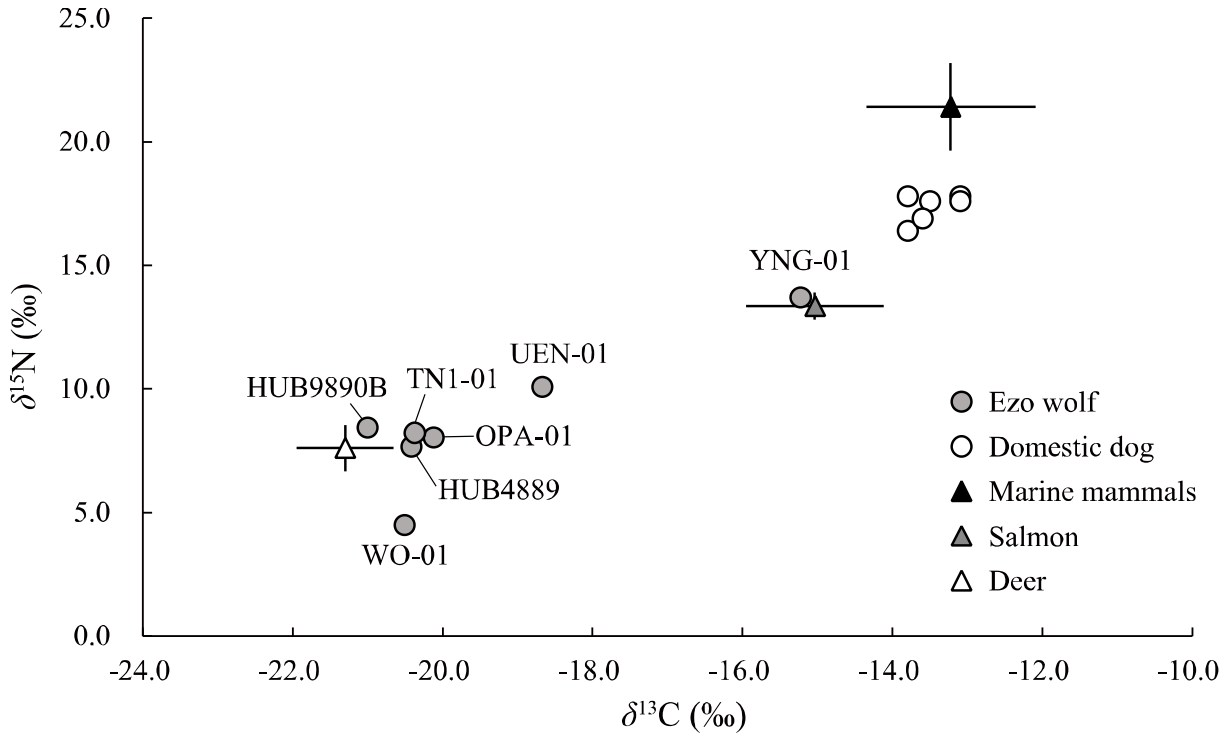


Fig. 1. Origins of the specimens on Hokkaido.



331

332 Fig. 2. Carbon and nitrogen stable isotope ratios in bone collagen of Ezo wolves, domestic

333 dogs (Naito et al. 2010, Tsutaya et al. 2014), and potential prey (mean ± SD). Ratios of prey

334 species have been corrected to account for isotope discrimination factors.

335

Table 1. Summary of carbon and nitrogen isotope analyses of faunal bones, and results of the stable isotope mixing models for estimation of the diet of individual wolves.

ID	Group	δ^{13}C (‰)	δ^{15}N (‰)	%C	%N	C/N
HUB9889	Wolf	-20.4	7.7	46.18	15.99	3.37
HUB9890B	Wolf	-21.0	8.4	44.12	15.70	3.28
OPA-01	Wolf	-20.1	8.0	42.01	14.24	3.44
TN1-01	Wolf	-20.4	8.2	40.91	14.06	3.39
UEN-01	Wolf	-18.7	10.1	39.88	13.77	3.38
WO-01	Wolf	-20.5	4.5	41.20	14.66	3.28
YNG-01	Wolf	-15.2	13.7	45.08	14.68	3.58
TKR-01	Wolf [a]	*-22.9*	*11.9*	*22.85*	*3.01*	*8.86*
	Deer [b]	-22.4 ± 0.6	3.7 ± 0.9	39.2 ± 3.3	13.8 ± 1.3	3.3 ± 0.0
	Salmon [b]	-162 ± 0.9 [c]	9.5 ± 0.5 [c]	42.1 ± 1.6	15.6 ± 0.7	3.2 ± 0.1
	Marine mammals [b]	-14.4 ± 1.1	17.5 ± 1.8	38.0 ± 3.7	13.5 ± 1.4	3.3 ± 0.0

a. This sample showed an unacceptably high C/N ratio.

b. Mode ± high and low 95 % credible intervals.

c. After correction for temporal isotopic shifts.

342 Table 2. Results of stable isotope mixing models for estimation of the diet of individual wolves.

ID	Deer	Salmon	Marine mammals
HUB9889	86.6 (75.1–96.7)	3.8 (0.0–20.0)	1.1 (0.0–13.2)
HUB9890B	88.9 (78.3–97.8)	1.4 (0.0–16.7)	1.1 (0.0–12.5)
OPA-01	82.3 (70.9–94.8)	6.3 (0.0–23.8)	1.4 (0.0–15.0)
TN1-01	84.5 (72.8–95.6)	5.1 (0.0–21.9)	1.2 (0.0–14.6)
UEN-01	61.6 (46.9–76.1)	31.1 (1.8–48.5)	2.0 (0.0–25.8)
WO-01	94.3 (85.0–99.3)	1.1 (0.0–11.9)	0.7 (0.0–7.7)
YNG-01	21.2 (1.6–34.4)	44.7 (17.4–89.5)	33.9 (2.5–52.1)

343 Values are mode ± high and low 95% credible intervals.

344

345 Table 3. Radiocarbon dates and estimated age of each Ezo wolf or archaeological site.

ID	Site	Common name	Scientific name	pMC (%)	^{14}C age (yrBP)	Calibrated age
HUB9889	Hokkaido Univ.	Wolf	*Canis lupus hattai*	-	-	1879 AD
HUB9890B	Hokkaido Univ.	Wolf	*Canis lupus hattai*	-	-	1881 AD
OPA-01	Opaushinai	Wolf	*Canis lupus hattai*	-	-	1667-1913 AD
TN1-01	Tenneru 1	Wolf	*Canis lupus hattai*	56.71 ± 0.20	4560 ± 30	3483 - 3106 BC
UEN-03	Uenae	Deer	*Cervus nippon*	55.11 ± 0.20	4790 ± 30	3643 - 3521 BC
WO-01	Wakkaoi	Wolf	*Canis lupus hattai*	53.11 ± 0.19	5080 ± 30	3961 - 3798 BC
YNG-02	Yanagidate	Deer	*Cervus nippon*	53.12 ± 0.19	5080 ± 30	3960 - 3798 BC

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